AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1. (Currently amended) A method for determining an HIV-1 subtype~~subtypes~~ comprising, ~~characterized by comprising the steps of~~

amplifying a polynucleotide comprising~~nucleic acid using as a target sequence~~ a portion ~~of a nucleotide sequence of the env gene~~ of an HIV-1 env gene to obtain an amplification product, wherein said amplification product is indicative of one of four HIV-1 subtypes at~~where at least one of~~ the 5' terminal and/or 3' terminal region~~nucleotide sequences is different depending on the HIV-1 subtype, and~~,

detecting said amplification product, thereby determining the HIV-1 subtype~~depending on whether or not the nucleic acid has been amplified~~.

2. (Currently amended) The method according to Claim 1, wherein said amplification product~~the target sequence~~ is between 100 and~~to~~ 2500 nucleotides long.

3. (Currently amended) The method according to Claim 1, wherein said~~the sequence from the 1st through 30th bases from the~~ ~~3~~5' terminal and/or ~~5~~3' terminal region is between 1 and 30 nucleotides long~~of the target sequence is different depending on the subtype~~.

4. (Currently amended) The method according to Claim 3, wherein saidthe 3' terminal regionof the target sequence is in athe C3 region of saidthe HIV-1 env gene of HIV-1.

5. (Currently amended) The method according to Claim 4, wherein saidthe 5' terminal regionof the target sequence is in athe C2 region of saidthe HIV-1 env gene of HIV-1.

6. (Currently amended) The method according to Claim 1, wherein multipledifferent amplification reactions are conductedcarried out, wherein each of said multiple amplification reactions use a using different primer pairpairs of primers, and wherein each primer pair amplifies a polynucleotide of aand different HIV-1 subtypesubtypes are detected.

7. (Currently amended) The method according to Claim 6, wherein at least two different HIV-1 subtypes are determineddetected by conducting at least two reactionscarrying out amplification at least twice with different primer pairs, wherein each of said of primers using primer pairs consistsing of a first primer comprising(primer 1) that includes a nucleotide sequence complementary to a portion of a C3 region of the HIV-1 env geneportion of the nucleotide sequence (nucleotide sequence 1) that is indicative of one of four HIV-1 subtypesdiffers depending on subtype in the C3 region of the env gene of HIV-1, and a second primer (primer 2) that comprisesincludes a nucleotide sequence complementary to a portion of a C2 region of the HIV-1 env geneportion of the nucleotide sequence (nucleotide sequence 2) of the C2 region of the env gene of HIV-1.

8. (Currently amended) The method according to Claim 1, wherein said amplifying is conducted using nested PCR~~a first amplification reaction is carried out with a first pair of primers using as a target sequence a portion of a nucleotide sequence of the env gene of HIV-1, a second amplification reaction is then carried out with a second pair of primers using as a target sequence a portion of said nucleotide sequence, where at least one of the 5' terminal and 3' terminal nucleotide sequences is different depending on the HIV-1 subtype, and the subtype is detected depending on whether or not the nucleic acid has been amplified by the second amplification reaction.~~

9. (Currently amended) The method according to Claim 8, wherein said nested PCR comprises at least two amplification steps, a first step and a second step,

wherein said second step uses a primer pair that includes~~the second pair of primers consists of~~ a first primer comprising~~(primer 1) that includes~~ a nucleotide sequence complementary to a portion of a~~the~~ C3 region of the HIV-1 env gene~~nucleotide sequence (nucleotide sequence 1)~~ that is indicative of one of four~~differs depending on subtype in the C3 region of the env gene of~~ HIV-1 subtypes, and a second primer ~~(primer 2)~~ that comprises~~includes~~ a nucleotide sequence complementary to a portion of ~~the~~ a portion of a C2 region of the HIV-1 env gene~~nucleotide sequence (nucleotide sequence 2) of the C2 region of the env gene of HIV-1~~; and

wherein said first step uses at least two primers including~~the first pair of primers consists of~~ a third primer ~~(primer 3) that includes~~ comprising a nucleotide sequence complementary to ~~a portion of~~ a ~~nucleotide~~ sequence downstream~~(nucleotide sequence 3)~~ of said portion of a C3 region of the HIV-1 env gene~~a region downstream of the 3' terminal of nucleotide sequence 1 of the env gene of HIV-1~~, and a fourth primer ~~(primer 4) that includes~~ comprising a nucleotide sequence complementary to a sequence upstream of said~~a~~ portion of a C2 region of the HIV-1 env gene ~~nucleotide sequence (nucleotide sequence 4) of a region upstream of the 5' terminal of nucleotide sequence 2 of the env gene of HIV-1~~.

10. (Currently amended) The method according to Claim 8, wherein said nested PCR comprises a first step and a second step, wherein said second step comprises at least two separate reactions each conducted with a different primer pair, and wherein each of said different primer pairs are indicative of one of four HIV-subtypes~~at least two subtypes are distinguished by repeating at least once, with different pairs of second primers, a series of operations comprising: a first amplification reaction that is carried out with the first pair of primers using as a target sequence a portion of a nucleotide sequence of the env gene of HIV-1; a second amplification reaction that is then carried out with the second pair of primers using as a target sequence a nucleotide sequence within said target sequence; and the detection of subtypes depending on whether or not the nucleic acid has been amplified by the second amplification reaction.~~

11. (Currently amended) A method for determining HIV-1 subtype comprising, conducting at least four nested PCR reactions~~The method according to Claim 10, wherein subtypes A, B, C, and E are distinguished by~~:

(a) wherein in a first nested PCR reaction which allows for determining~~detecting~~ HIV-1 subtype A, the following primers are used in step 1: ~~using as the first primer pair a mixture of~~ primer 12A represented by~~containing nucleotide sequence GCAATAGAAAAATTCTCCTC~~ (Sequence ID No. 5~~),~~ ~~and~~ primer 12B represented by~~containing nucleotide sequence ACAGTAGAAAAATTCCCCTC~~ (Sequence ID No. 6~~)~~, ~~and a mixture of~~ primer 9AE represented by~~containing nucleotide sequence CACAGTACAATGCACACATG~~ (Sequence ID No. 8~~)~~, and primer 9B represented by~~containing nucleotide sequence CACAGTACAATGTACACATG~~ (Sequence ID No. 9~~)~~, and wherein the following primers are used in step 2 of said first nested PCR reaction: ~~using as the second primer pair~~ primer 11QA1 represented by ~~containing nucleotide sequence CTCCTGAGGAGTTAGCAAAG~~ (Sequence ID No. 27~~)~~, and primer 10U represented by~~containing nucleotide sequence CTGTTAAATGGCAGTCTAGC~~ (Sequence ID No. 20~~)~~;

(b) wherein in a second nested PCR reaction which allows for determining HIV-1 subtype B, the following primers are used in step 1:~~detecting subtype B using as the first primer pair a mixture of~~ primer 12A represented by~~containing nucleotide sequence GCAATAGAAAAATTCTCCTC~~ (Sequence ID No. 5~~),~~ ~~and~~ primer 12B represented by~~containing nucleotide sequence ACAGTAGAAAAATTCCCCTC~~ (Sequence ID No. 6~~)~~, ~~and a mixture of~~ primer 9AE represented ~~containing nucleotide sequence~~

~~CACAGTACAATGCACACATG (~~Sequence ID No. 8~~),~~ and primer 9B represented by~~containing nucleotide sequence CACAGTACAATGTACACATG (~~Sequence ID No. 9~~)~~, and wherein the following primers are used in step 2 of said second nested PCR reaction:~~and using as the second primer pair~~ primer 11VB represented by~~containing nucleotide sequence CACAATTAAAACTGTGCATTAC (~~Sequence ID No. 28) and primer 10U represented by~~containing nucleotide sequence CTGTTAAATGGCAGTCTAGC (~~Sequence ID No. 20);

(c) wherein in a third nested PCR reaction which allows for determining HIV-1 subtype C, the following primers are used in step 1:~~detecting subtype C using as the first primer pair a mixture of~~ primer 12A represented by ~~containing nucleotide sequence GCAATAGAAAAATTCTCCTC (~~Sequence ID No. 5~~),~~ and primer 12B represented by ~~containing nucleotide sequence ACAGTAGAAAAATTCCCCTC (~~Sequence ID No. 6~~),~~ and a mixture of primer 9AE represented by~~containing nucleotide sequence CACAGTACAATGCACACATG (~~Sequence ID No. 8~~),~~ and primer 9B represented by~~containing nucleotide sequence CACAGTACAATGTACACATG (~~Sequence ID No. 9~~)~~, and wherein the following primers are used in step 2 of said third nested PCR reaction:~~using as the second primer pair~~ primer 11XC represented by ~~containing nucleotide sequence TTGTTTTATTAGGGAAGTGTTC (~~Sequence ID No. 29~~),~~ and primer 10UC represented by~~containing nucleotide sequence CTGTTAAATGGTAGTCTAGC (~~Sequence ID No. 24); and

(d) wherein in a fourth nested PCR reaction which allows for determining HIV-1 subtype E, the following primers are used in step 1: ~~detecting subtype E using as the first primer pair a mixture of~~ primer 12A represented by~~containing nucleotide sequence~~

~~GCAATAGAAAAATTCTCCTC (~~Sequence ID No. 5~~)~~ ~~,and~~ primer 12B represented by~~containing nucleotide sequence ACAGTAGAAAAATTCCCCTC (~~Sequence ID No. 6~~)~~, ~~and a mixture of~~ primer 9AE represented by~~containing nucleotide sequence CACAGTACAATGCACACATG (~~Sequence ID No. 8~~)~~, and primer 9B represented by~~containing nucleotide sequence CACAGTACAATGTACACATG (~~Sequence ID No. 9~~)~~, and wherein the following primers are used in step 2 of said fourth nested PCR reaction:~~using as the second primer pair~~ primer 11WE represented by~~containing nucleotide sequence CTCTACAATTAAAATGATGCATTG (~~Sequence ID No. 30~~,)~~ and primer 10U represented by ~~containing nucleotide sequence CTGTTAAATGGCAGTCTAGC (~~Sequence ID No. 20~~)~~.

12. (Original) The method according to Claim 8, wherein at least two subtypes are distinguished by repeating at least once, with different pairs of first and second primers, a series of operations comprising: a first amplification reaction that is carried out with a first pair of primers using as a target sequence a portion of a nucleotide sequence of the env gene of HIV-1; a second amplification reaction that is then carried out with a second pair of primers using as a target sequence a nucleotide sequence within said target sequence; and the detection of subtypes depending on whether or not the nucleic acid has been amplified by the second amplification reaction.

13. (Currently amended) A~~The~~ method for determining HIV-1 subtype comprising, conducting at least three nested PCR reactions:~~according to Claim 12, wherein subtypes A, B, and E are distinguished by~~:

(a) wherein in a first nested PCR reaction which allows for determining HIV-1 subtype A, the following primers are used in step 1: ~~detecting subtype A using as the first primer pair~~ primer 12A represented by ~~containing nucleotide sequence GCAATAGAAAAATTCTCCTC~~ (Sequence ID No. 5) and primer 9AE represented by ~~containing nucleotide sequence CACAGTACAATGCACACATG~~ (Sequence ID No. 8), wherein the following primers are used in step 2 of said first nested PCR reaction:~~and using as the second primer pair~~ primer 11QA represented by ~~containing nucleotide sequence CTCCTGAGGGGTTAGCAAAG~~ (Sequence ID No. 1~~)~~ and primer 10 represented by~~containing nucleotide sequence AAATGGCAGTCTAGCAGAAG~~ (Sequence ID No. 4~~)~~;

(b) wherein in a second nested PCR reaction which allows for determining HIV-1~~detecting~~ subtype B, the following primers are used in step 1:~~ using as the first primer pair~~ primer 12B represented by~~containing nucleotide sequence ACAGTAGAAAAATTCCCCTC~~ (Sequence ID No. 6~~)~~ and primer 9B represented by~~containing nucleotide sequence CACAGTACAATGTACACATG~~ (Sequence ID No. 9~~)~~, wherein the following primers are used in step 2 of said second nested PCR reaction:~~and using as the second primer pair~~ primer 11BB represented by~~containing nucleotide sequence CTGTGCATTACAATTTCTGG~~ (Sequence ID No. 2~~)~~ and primer 10 represented by~~containing nucleotide sequence AAATGGCAGTCTAGCAGAAG~~ (Sequence ID No. 4~~)~~; and

(c) wherein in a third nested PCR reaction which allows for determining HIV-1~~detecting~~ subtype E, the following primers are used in step 1:~~using as the first primer pair~~ primer 12E represented by ~~containing nucleotide sequence GCAATAGAAAAATTCCCCTC~~ (Sequence ID No. 7~~)~~ and primer 9AE represented by ~~containing nucleotide sequence CACAGTACAATGCACACATG~~ (Sequence ID No. 8~~)~~, and wherein the following primers are used in step 2 of said third nested PCR reaction:~~using as the second primer pair~~ primer 11QE represented by~~containing nucleotide sequence CTCCTGAGGGTGGTTGAAAG~~ (Sequence ID No. 3~~)~~ and primer 10 represented by~~containing nucleotide sequence AAATGGCAGTCTAGCAGAAG~~ (Sequence ID No. 4~~)~~.

14. (Currently amended) A method for determining the presence or absence of HIV-1 in a sample~~The method according to Claim 1, further~~ comprising, ~~the steps of~~

amplifying an HIV-1 polynucleotide from said sample~~nucleic acid using as a target sequence a portion of a nucleotide sequence of the HIV-1 genome~~, wherein said HIV-1 polynucleotide is~~the nucleotide sequence being~~ highly conserved among all HIV-1 subtypes, and

determining~~ascertaining~~ the presence or absence of HIV-1 in said sample, wherein HIV-1 is present in said sample where said polynucleotide is~~depending on whether or not the nucleic acid has been~~ amplified.

15. (Currently amended) The method according to Claim 14, wherein said amplifying is conducted using nested PCR.~~the step for ascertaining the presence or absence of~~

~~HIV-1 comprises amplifying the nucleic acid with a first primer pair using as a target sequence a portion of a nucleotide sequence of the HIV-1 genome, the nucleotide sequence being highly conserved among all subtypes, then carrying out a second amplifying reaction with a second primer pair using as a target sequence a nucleotide sequence in said target sequence, and ascertaining the presence or absence of HIV-1 depending on whether or not the nucleic acid has been amplified.~~

16. (Currently amended) The method according to Claim 15, wherein ~~the~~ said amplifying uses~~primers that are used comprise a mixture of~~ a plurality of upstream primers with differen~~ting~~ nucleotide sequences and a plurality of downstream primers with differen~~ting~~ nucleotide sequences.

17. (Currently amended) The method according to Claim 16, wherein said nested PCR comprises two steps:

wherein the following primers are used in step 1: ~~the first primers comprise a mixture of~~ primer 12A represented by~~containing nucleotide sequence GCAATAGAAAAATTCTCCTC~~ ~~(~~Sequence ID No. 5~~)~~, primer 12B represented by ~~containing nucleotide sequence ACAGTAGAAAAATTCCCCTC~~ (Sequence ID No. 6~~)~~, primer 9AE represented by ~~containing nucleotide sequence CACAGTACAATGCACACATG~~ (Sequence ID No. 8~~)~~, and primer 9B represented by ~~nucleotide sequence CACAGTACAATGTACACATG~~ (Sequence ID No. 9), and

wherein the following primers are used in step 2: ~~the second primer pair comprises~~ primer 11LB represented by ~~containing nucleotide sequence AATTTCTGGGTCCCCTCCTG~~ ~~(~~Sequence ID No. 18~~)~~, primer 11LAE represented by~~containing nucleotide sequence~~ ~~AATTTCTAGATCCCCTCCTG (~~Sequence ID No. 25~~)~~, primer 11LC represented by ~~containing nucleotide sequence AATTTCTAGGTCCCCTCCTG (~~Sequence ID No. 26~~)~~, and primer 10U represented by~~containing nucleotide sequence CTGTTAAATGGCAGTCTAGC (~~Sequence ID No. 20~~)~~.

18. (Currently amended) A kit for determining HIV-1 subtypes~~subtypes~~, comprising polynucleotide primers ~~pairs~~ capable of amplifying~~in which a target sequence is~~ a portion of a polynucleotide~~nucleotide sequence~~ of ~~the~~ HIV-1 env gene ~~of HIV-1~~, wherein said polynucleotide is indicative of one of four HIV-1 subtypes at ~~where at least one of~~ the 5' terminal and/or 3' terminal region of said polynucleotide~~nucleotide sequences is different depending on the subtype~~.

19. (New) A method for determining an HIV-1 subtype comprising,

amplifying a polynucleotide from a sample containing HIV-1 DNA, wherein said polynucleotide is amplified in a reaction comprising at least two nucleotide primers that comprise the nucleotide sequences represented by SEQ. ID NOS. 20 and 28,

designating said HIV-1 DNA as HIV-1 subtype B if said polynucleotide is amplified.

20. (New) A kit for determining HIV-1 subtype comprising, at least two primers comprising the nucleotide sequences represented by SEQ. ID NOS. 20 and 28.